g) once a day for 5 days, nine received placebo. At day 14, animals were sacrificed and angiogenesis analysed by counting vessels on histological sections.
RESULTS (shown in the table below): CD 31 quantification pointed out a minimal angiogenesis induced in control implants, significantly increased in bFGF implants, and surprisingly enhanced in bFGF+ASIS implants. However, TF inhibition significantly decreased the number of perfused vessels, as shown by CD31 perfused vessels quantification and May Grunwald Giemsa quantification.
CONCLUSION: TF inhibition does not inhibit bFGF-induced angiogenesis, but rather impairs neovascularization maturation, with a dramatical decrease of perfused bFGF-induced vessels. These results indicate the critical role of TF in post-natal non-tumoral angiogenesis.

	CD31 total vessels	CD31 perfused vessels	May Grunwald Giemsa
control (n=5)	70+/20	64+/-13	42+/-27
bFGF + placebo (n=9)	140+/-34 *	104+/-35	104+/-26
bFGF+ ASIS (n=8)	182+/-66 *	59+/-9 †	56+/-16 †

*P<0.0001 vs placebo. †P<0.0001 vs bFGF

1008-75 A Growth Factor Mixture That Significantly Enhances Collateral Growth

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Background: Studies of therapeutic angiogenesis have focused on use of single growth factors. However, angiogenesis is complex and multiple factors participate on the cascade. Moreover, recent publications have identified synergistic effects of angiogenic agents in the induction of vascular growth. We tested the efficacy of a naturally occurring growth factor mixture (GFm) isolated from bovine bones and previously shown to stimulate angiogenesis more potently than single growth factors.
Methods: Components of GFm (SDS-PAGE, mass spectrometry and Western blot) include transforming growth factor beta 1-3, bone morphogenic protein 2-7, and fibroblast growth factor 1. To evaluate the angiogenic potential, a randomized, blinded, placebo-controlled study was performed. An LAD ameroid constrictor was placed in 21 dogs to create chronic ischemia. 3 weeks later, angiography confirmed ameroid closure and defined the degree of collateral filling of the distal LAD. Dogs were randomized into 3 groups: GFm1 (1 mg/ml), GFm10 (10 mg/ml) or placebo (P), each injected into ischemic myocardium (15-20 injections/heart, 0.15 ml/injection) via thoracotomy. Animals received bromodeoxyuridine (BrdU) injections to label proliferating cells. 6 weeks later angiography studies were repeated. Hearts were removed and evaluated histologically. Histology and angiograms were graded with semiquantitative scales. All analyses were done blinded to treatment group.
Results: Histologically, GFm dose dependently induced growth of large, BrdU positive vessels (vascular growth index: 0.2±0.2, 1.0±0.2, 1.7±0.2 in P, GFm1 and GFm10, respectively; p=0.001). Angiographically, opacification of the distal LAD significantly improved with GFm (distal LAD opacification score: 0.4±0.2, 1.1±0.14, 1.6±0.3 in P, GFm1 and GFm10, respectively; p=0.014).
Conclusion: GFm is a potent angiogenic agent that stimulates large vessel growth in chronically ischemic myocardium in a dose dependent manner. Collateral vessels developed to the occluded LAD to significantly improve or completely reconstitute its visualization during contrast injection.

1008-76 Trans-Epicardial Transplantation of Mature Endothelial Cells Induces Neoangiogenesis and Improves LV Function in an Animal Model of Ischemic Cardiomyopathy

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Objective: We hypothesize that transplantation of heterotopic mature endothelial cells (EC) within a fibrin platform would increase production of endogenous growth factors and promote development of a functional capillary network. The objective of this study was to investigate the effect of autologous EC transplantation using a fibrin meshwork on ischemic myocardium of sheep.
Methods: Placing ameroid constrictors on the circumflex artery of 12 adult sheep produced ischemic cardiomyopathy. Three groups were randomized (4 animals/group) to transepicardial injection (3 sites/animal), 4 weeks after ameroid placement: sham treatment (saline with denatured cells), controls (no injections), and EC fibrin meshwork (autologous mature EC, $5x10^5$ per injection site, harvested from jugular veins). Eight weeks after trans-epicardial treatment animals were assessed for myocardial function (echo), myocardial flow, histology (capillary area) and myocardial ultrastructure (TEM).
Results: Eight weeks after EC/sham/control treatment, EF was markedly improved in the EC transplant group (0.56+-0.04 p=0.029 versus baseline), whereas in the sham and control groups, EF deteriorated (0.40+-0.08 p= 0.013 Vs EC and 0.39+-0.05 p=0.0017 Vs EC group respectively). Myocardial blood flow (ml/min/g) measured with colored microspheres was also significantly increased in the EC transplant group (0.66+-0.10 versus 0.15+-0.03 in control and 0.18+-0.05 in saline group; p=0.0009 Vs control and p=0.0001 Vs saline). In the EC transplant zones, histology revealed extensive neovascularization (significant increase in capillary area) and TEM demonstrated functional arterioles and cappilaries.
Conclusion: Trans-epicardial heterotopic transplantation of EC within a fibrin matrix dramatically enhances neovascularization, increases myocardial blood flow and improves LV function in an animal model of ischemic cardiomyopathy. The fibrin meshwork platform may serve as a generalized enhancement vehicle for other stem cell transplant methodologies to treat pts with refractory ischemia and/or LV dysfunction.

1008-77 Long-Term Safety and Efficacy of Intramuscular Administration of CI-1023 (AdGVVEGF121.10) in Patients With Intermittent Claudication

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Background: In a Phase I study, we evaluated the safety and efficacy of CI-1023 (Ad_{GV}VEGF121.10, adenovirus encoding vascular endothelial growth $factor_{121}$) in patients with advanced intermittent claudication (IC) secondary to SFA/infrageniculate disease.
Methods: Patients were randomized to IM dosing of placebo (n=3) or CI-1023 (4×10^8 to 4×10^{10} particle units, n= 15).
Results: 73% and 33% of CI-1023 and placebo patients respectively completed 1 year of follow-up. Edema and rash were the most common early adverse event with 47% of CI-1023 subjects developing edema (mild- moderate) in the injected extremity. One infrainguinal bypass procedure occurred in the placebo and CI-1023 groups at day 29 and 104 respectively. One death (day 160) and one malignancy (day 274) occurred in the CI-1023 group, none were judged related to CI-1023. Table 1 summarizes the median change in peak walking time (PWT), claudication onset time (COT) and ankle brachial indices (ABI) for the CI-1023 group.

	30 days	90 days	180 days	360 days
Δ Rest *ABI*	0.04, 0.11 (14)	0.21*, 0.13(6)	0.09*, 0.30 (12)	0.09*, 0.06 (9)
Δ Stress ABI	-0.06, 0.13 (7)	0.02, 0.06 (4)	-0.09, 0.13 (6)	-0.08, 0.10 (4)
Δ COT	0.5, 0.8 (4)	0.2, 1.8 (2)	0.1, 1.1 (3)	0.5, 0 (1)
Δ PWT	1.0*, 0.7 (8)	2.0, 5.0 (4)	2.0, 4.1 (7)	4.8, 5.5 (4)

All values are Median, Inter-quartile range (75^{th} - 25^{th} percentile). Parenthesis = Number of patients with data at baseline and time point. * $p < 0.05$ by signed rank test
The walking distance perception score in the walking impairment questionnaire showed an improvement at 30 days corresponding to the improvement in PWT.
Conclusions: Administration of CI-1023 to IC patients was adequately tolerated and associated with an apparent improvement in clinical outcomes upto 1 year. Larger studies with placebo controls in IC are ongoing.

1008-78 Induction of Angiogenesis by Metalloproteinase Derived From Venom of Indian Cobra in a Model of Rat Hind Limb Ischemia

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Background: The field of therapeutic angiogenesis has evolved from formation of capillary sprouts to development of arterial collaterals (arteriogenesis). One of the shortcomings of strategies designed solely to stimulate proliferation of endothelial cells (ECs) such as VEGF therapy, is the fact that only capillary (<40μm) networks are formed. Instead, formation of large conductance arterioles require a series of sequential steps involving EC migration, adhesion and differentiation and smooth muscle cell (SMC) migration. Venom from Indian cobra contains several potent enzymes including metalloproteinases (MP). We undertook the present study to test efficacy of MP and phospholipase A2 (PLA-2) derived from venom of Indian cobra and compare it with plasmid VEGF 165 (ph VEGF 165) gene.
Methods : Male Wister albino rats (6 in each group) were randomized to receive MP 1mg/kg, PLA-2 2.5mg/kg, ph VEGF 165 400μg or normal saline (control), 2 days after left femoral artery division. Fifteen days after femoral artery division, digital subtraction angiography (DSA) was performed and muscles of thigh distal to the site of division subjected to histopathological examination.
Results : On DSA there was a mild increase in number of large arterial collaterals both in MP and pH VEGF 165 groups as compared to PLA-2 or saline groups (statistically not significant). Histopathological examination revealed that there was a marked stimulation of neo-angiogenesis in the MP group and ph VEGF group as compared to PLA-2 or the saline group. Capillary density score was : saline, 1.1 ± 1.0; PLA-2, 1.2 ± 1.0; MP, 2.0 ± 0.9 (p<0.05 vs saline group) ph VEGF, 2.3 ± 1.1 (p<0.05 vs saline group). There was a marked inflammatory response in the injected muscles in the MP group.
Conclusions : Metalloproteinase derived from venom of Indian cobra can lead to profound angiogenesis comparable to that produced by VEGF gene therapy. Further studies are warranted to confirm its efficacy and to evaluate the role of co-administration with other angiogenic agents.